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Identification of *Jade1*, a Gene Encoding a PHD Zinc Finger Protein, in a Gene Trap Mutagenesis Screen for Genes Involved in Anteroposterior Axis Development

Elena Tzouanacou,[†] Susan Tweedie, and Valerie Wilson*

Institute for Stem Cell Research, University of Edinburgh, Edinburgh EH9 3JQ, United Kingdom

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In a gene trap screen for genes expressed in the primitive streak and tail bud during mouse embryogenesis, we isolated a mutation in *Jade1*, a gene encoding a PHD zinc finger protein previously shown to interact with the tumor suppressor pVHL. Expressed sequence tag analysis indicates that *Jade1* is subject to posttranscriptional regulation, resulting in multiple transcripts and at least two protein isoforms. The fusion *Jade1*– β -galactosidase reporter produced by the gene trap allele exhibits a regulated expression during embryogenesis and localizes to the nucleus and/or cytoplasm of different cell types. In addition to the primitive streak and tail bud, β -galactosidase activity was found in other embryonic regions where pluripotent or tissue-specific progenitors are known to reside, including the early gastrulation epiblast and the ventricular zone of the cerebral cortex. Prominent reporter expression was also seen in the extraembryonic tissues as well as other differentiated cell types in the embryo, in particular the developing musculature. We show that the gene trap mutation produces a null allele. However, homozygotes for the gene trap integration are viable and fertile. Database searches identified a family of Jade proteins conserved through vertebrates. This raises the possibility that the absence of phenotype is due to a functional compensation by other family members.

Patterning of the mammalian embryo along the anteroposterior (A/P) axis involves complex morphogenetic and tissue diversification events taking place during gastrulation and organogenesis in the primitive streak and tail bud. The appearance of the streak on the prospective posterior side of the embryo at the onset of gastrulation (6.5 days postcoitum [d.p.c.]) constitutes the first morphological asymmetry indicating the polarity of the A/P axis. In the streak, cells lose contact with the epiblast (primitive ectoderm) and migrate anteriorly to give rise to the mesoderm and definitive endoderm. The axis is laid down progressively in a rostrocaudal sequence. Early during gastrulation, progenitors for the axial tissues are located throughout the epiblast (26). At later stages, however, lineage analysis studies support the existence of a resident pool of progenitors in the streak and its descendant, the tail bud, which can maintain itself and give rise to the entire postcranial axis (4, 35, 52, 63). Several genes expressed in these regions were shown to be involved in maintenance of progenitor populations, specification, and patterning of mesoderm or morphogenetic movements (reviewed in reference 53). One such gene is *T* (*Brachyury*). Heterozygotes for a loss of function mutation in *T* have short or absent tails (7), whereas homozygotes die at midgestation lacking structures posterior to the forelimb (3, 66). *T*, the founder member of the T-box family of transcription factors, is one of the earliest markers of nascent mesoderm. It is expressed in the streak during gastrulation and in

the tail bud until the end of axial elongation at 13.5 d.p.c (3, 64). Thus, the primitive streak and tail bud play a pivotal role in the formation and patterning of axial tissues. However, the basic mechanisms underlying development of the axis are still not clearly understood, and the genes directing these processes remain largely unknown.

Gene trapping is an attractive method for creation of insertional mutations in embryonic stem (ES) cells. This is specifically designed to enrich for intragenic integration events, since it makes use of promoterless reporter constructs that need to integrate downstream of a gene's regulatory sequences to activate reporter expression (reviewed in reference 49). Gene trap insertions result in production of fusion transcripts consisting of the reporter and upstream endogenous sequences. Thus, mutated genes can be readily identified by methods such as 5' rapid amplification of cDNA ends (5' RACE) or inverse PCR (55, 57). Reporter expression can be monitored in ES cell-derived chimeric or transgenic embryos, and in the majority of cases examined, it accurately reflects the expression pattern of the trapped gene. Moreover, the availability of ES cells and the development of techniques for their differentiation allows for in vitro preselection of integrations in genes expressed in specific lineages or responding to specific cues (10, 11, 59).

We undertook a gene trap screen in differentiating ES cells to identify novel genes expressed in the primitive streak and tail bud by screening in vitro for coexpression of the gene trap reporter and *T*. In this report, we describe the characterization of a gene trap insertion into a mouse gene encoding a PHD zinc finger protein. A human cDNA clone, *Jade-1*, corresponding to a short transcript of this gene, was recently identified in a two-hybrid screen for proteins interacting with pVHL (von Hippel-Lindau syndrome protein) tumor suppressor (69). During embryogenesis, the fusion *Jade1*– β -galactosidase (β -Gal)

* Corresponding author. Mailing address: Institute for Stem Cell Research, University of Edinburgh, King's Buildings, Roger Land Building, West Mains Rd., EH9 3JQ Edinburgh, United Kingdom. Phone: 44 131 6506424. Fax: 44 131 6507773. E-mail: V.Wilson@ed.ac.uk.

[†] Present address: Unité de Biologie Moléculaire du Développement, Institut Pasteur, 75724 Paris Cedex 15, France.

reporter showed a restricted expression pattern, in particular in extraembryonic tissues and in embryonic regions known to harbor pluripotent or tissue-specific progenitors. We show that integration of the gene trap vector generates a null allele of *Jade1*. However, mice homozygous for this allele do not show any obvious phenotypic defects.

MATERIALS AND METHODS

Culture, electroporation, and screening of gene trap ES cell clones. E14TG2a ES cells (17) were cultured as described in reference 48, except that they were grown in Glasgow's minimal essential medium supplemented with 0.25% sodium bicarbonate, 0.1% nonessential amino acids, 4 mM glutamine, 2 mM sodium pyruvate, 0.1 M 2-mercaptoethanol, 10% heat-inactivated fetal calf serum, and 100 U of leukemia inhibitory factor (LIF)/ml. ES cells (10^8) were electroporated with a 150- μ g/ml equimolar mixture of pGT1,2,3 *Hind*III linearized vectors as described previously (64) and selected with 100 μ g of G418/ml for 10 days. Resistant clones were differentiated by removal of LIF for 72 h and screened by 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) staining and in situ hybridization with a *T* riboprobe (61) as described previously (51).

Production of transgenic mouse line, breeding, and genotyping. Chimeric mice were produced by injection of E148 ES cells into C57BL/6 blastocysts as described previously (41). F₁ animals heterozygous for the gene trap mutation *Jade1*^{gtE148ISCR} were obtained by mating male chimeras with C57BL/6 females. F₂ to F₅ heterozygotes (129/Ola:C57BL/6 mixed background) were intercrossed to generate homozygous individuals. All work with animals was carried out under United Kingdom Project license 60/2107.

Genotyping of animals was routinely performed by X-Gal staining of tail biopsy specimens. Homozygosity for the gene trap was initially determined by quantitative Southern blotting and analysis following identification of the trapped gene by PCR. Blots were probed with a *Bgl*II/*Bam*HI fragment corresponding to the *engrailed-2* intron by standard procedures (44), and the intensities of the endogenous *engrailed-2* gene (two-copy loading control) and gene trap vector-specific bands were compared. A common E148INT4 forward primer (5'-GATGTTAAGAGTGGCATCTCGG-3') and either E148INT5 (5'-ACATC TAGGAGTGGAACTAG-3') or pGT2rev (5'-CCACAACGGGTCTTCT GTTAG-3') reverse primer were used in separate PCRs to detect the wild-type or mutant *mJade1* alleles, respectively.

5' RACE-PCR, Northern blotting, and reverse transcription (RT)-PCR. Total RNA was extracted from cells or embryos by using Trizol reagent (Invitrogen) according to the manufacturer's instructions. 5' RACE-PCR was performed according to the method of Townley et al. (55). First-strand synthesis was primed with primer R1 (5'-TAATGGGATAGGTTACGT-3'). The product was poly(A) tailed, and primer R2 [5'-GGTGTGAGCTCTTCTAGATGG(T₁₇)-3'] was used in second-strand synthesis. First-round PCR was performed with primer R3 (5'-GGTGTGAGCTCTTCTAGATGG-3') and nested primer R4 (5'-AGTA TCGGCCTCAGGAAGATCG-3'). In second-round PCR, 5' biotin-R3 and R5 (5'-ATTGAGCTGCGCAACTGTTGG-3') primers were used. Second-round PCR products were directly sequenced with the Amplicycle sequencing kit (Perkin Elmer) with R6 (5'-GTTTCCCAGTCACGAC-3').

Northern blot hybridization was performed according to standard procedures (44) with 10 μ g of RNA. A 0.3-kb fragment complementary to the endogenous *mJade1* cDNA sequence, 3' to the gene trap integration site, was amplified by RT-PCR on RNA extracted from wild-type ES cells with primers E148/1 (5'-G ACCTGAAGATCGAAAGCCTTC-3') and mEST (5'-GATATCGACGTAGC CTAACGCT-3'), cloned into Topo-PCR2.1 vector (Invitrogen), and used to probe the blot.

RT-PCR on RNA extracted from 13.5-d.p.c. embryos was performed by using Superscript II (Invitrogen) according to the manufacturer's instructions. A poly(dT) primer was used for first-strand synthesis while fragments specific to wild-type or mutant transcripts were PCR-amplified with forward primer E148/2 (5'-GCAGCAGTGAGGATTCTGACGA-3') and reverse primer mEST (wild type) or R5 (*lacZ*, mutant).

X-Gal staining of whole-mount embryos and sections. Embryos were recovered and tested at different developmental stages. Noon of the day when a vaginal plug was detected was defined as 0.5 d.p.c. Embryos up to 9.5 d.p.c. were dissected in phosphate-buffered saline and stained with X-Gal as described in reference 2. Older embryos (10.5 to 12.5 d.p.c.) were treated and stained as described in reference 60. Cryosections (15 μ m thick) were processed and stained with X-Gal as young embryos (fixation time, 10 min). Paraffin sections of stained embryos were prepared as described in reference 22.

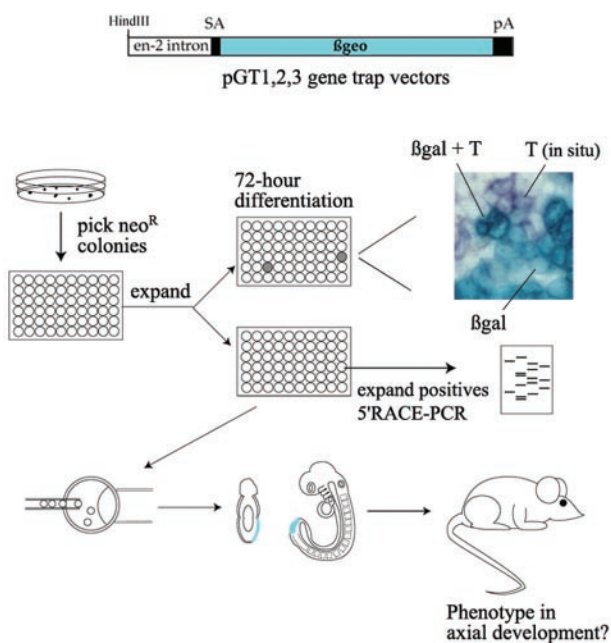


FIG. 1. Gene trap screen to identify mutations in genes expressed in primitive streak and tail bud. pGT1,2,3 vectors are introduced into ES cells, and G418^r colonies, representing integrations in the open reading frames, were selected. The different pGT vectors lack a translation initiation codon but allow selection of integrations in each of the three different reading frames. One set of duplicated colonies is screened for overlapping expression of β -Gal reporter activity (light blue) and *T* mRNA (purple) after in vitro differentiation, and positive clones are expanded from a master plate. After 5' RACE PCR to identify endogenous mRNA fused to the vector sequence, ES cell clones corresponding to bona fide integrations in the open reading frame of genes are injected into blastocysts to create chimeric embryos, where expression of β -Gal in the primitive streak is monitored. Lines testing positive are passed through the germ line to create transgenic mice which are used to extensively characterize reporter expression and examine embryonic or postnatal homozygous phenotypes.

Sequence analysis. The full-length *mJade1* mRNA (BN000281) encoding Jade1L, short *mJade1* mRNA for Jade1S (BN000282), and alternative noncoding exons (BN000283 and BN000284) were predicted from contigs of cDNAs and expressed sequence tags (ESTs) from unigene cluster Mm.28483. Additional family members in both mouse and other vertebrate species were identified by Blast searches. These sequences have also been submitted to the TPA database (BN000275 to BN000280 and BN000289). Multiple-sequence alignments and phylogenetic analyses were carried out with Clustalx. CpG islands and candidate promoters were identified by using CpG Island Searcher (<http://www.uscnorris.com/cpgislands/>) (56) and PROSCAN 1.7 (<http://bimas.dcrn.nih.gov/molbio/proscan/>).

Nucleotide sequence accession numbers. The following TPA database accession numbers were obtained in this study: full-length *mJade1* mRNA encoding Jade1L, BN000281; short *mJade1* mRNA encoding Jade1S, BN000282; alternative noncoding exons, BN000283 and BN000284; additional family members from mouse and other vertebrate species, BN000275 to BN000280 and BN000285 to BN000289.

RESULTS

A small-scale gene trap screen was performed following the electroporation of pGT1,2,3 vectors in ES cells (Fig. 1) (64). To enrich for integrations in genes expressed in the primitive streak and tail bud during embryogenesis, 279 neomycin-resistant clones were prescreened in vitro for cells coexpressing the

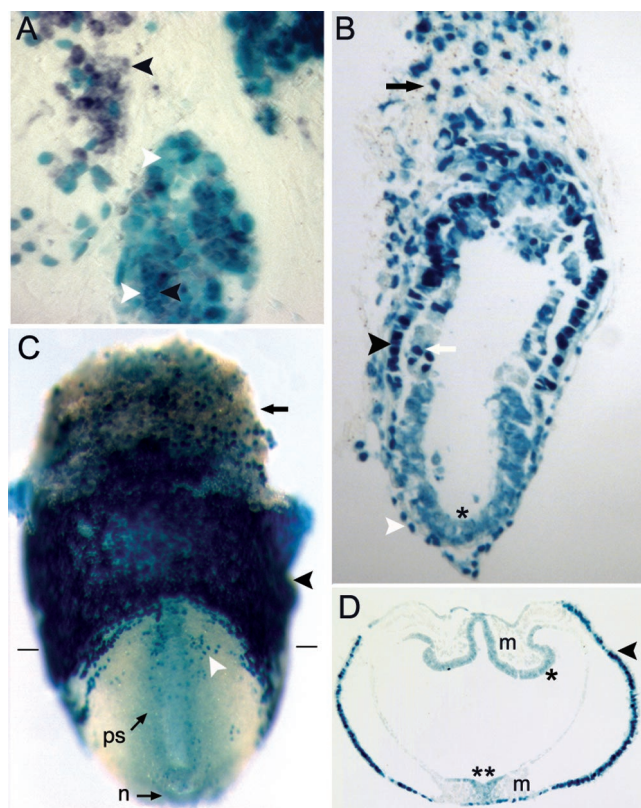


FIG. 2. Reporter expression in differentiating E148 ES cells and early embryos. (A) Partially overlapping expression of *T* mRNA (black arrowheads) and β -Gal (white arrowheads) in differentiating E148 ES cells. Approximately 40% of E148 β -Gal-positive cells also express *T*. β -Gal is localized in the nucleus. (B) Longitudinal section of a 6.5-d.p.c. embryo heterozygous for the E148 integration. β -Gal is strongly expressed in the extraembryonic visceral (black arrowhead) and parietal (white arrowhead) endoderm as well as in the extraembryonic ectoderm (white arrow) and trophoblast (black arrow). Cells in the epiblast (asterisk) express lower levels of β -Gal protein. (C) Posterior view of a whole-mount ~7.5-d.p.c. embryo, showing high level expression in the trophoblast (arrow) and visceral endoderm (black arrowhead) and lower expression in the primitive streak and node. Scattered cells (white arrowhead) in the embryonic endoderm express high levels of β -Gal protein. Horizontal lines indicate the approximate level and plane of the section shown in panel D. (D) Transverse section of the embryo shown in panel C, showing expression in the visceral endoderm and anterior neurectoderm (asterisk). In the primitive streak (double asterisk), cells in the ectoderm rapidly downregulate reporter expression after ingress to the adjacent mesodermal layer. ps, primitive streak; n, node; m, mesoderm.

gene trap reporter, *lacZ*, and *T*, marker of the streak and tail bud (Fig. 1). Expression of *T* was shown to be upregulated in patches of cells growing in monolayers during the first 96 h of differentiation in the absence of LIF, with a maximal increase between 48 and 72 h (47). Based on this observation, ES cell clones were subjected to differentiation for 72 h and double stained for expression of *lacZ*, with X-Gal, and *T* mRNA by in situ hybridization. β -Gal activity was detected in 191 clones (68%). One hundred sixty-eight clones showed nonubiquitous reporter expression, and of these, 26 exhibited various proportions of cells expressing both β -Gal and *T*. E148 was one of the clones presenting a high proportion of coexpressing cells and

was selected for further analysis (Fig. 2A). Chimeric embryos generated by injection of this clone into blastocysts and examined during gastrulation and organogenesis stages exhibited a restricted expression pattern including the primitive streak and tail bud (data not shown).

Reporter expression in E148 mice during embryogenesis. *lacZ* expression was examined in heterozygotes at various developmental stages. At 6.5 d.p.c., β -Gal activity was observed in all tissues but at various levels and in various subcellular compartments (Fig. 2B). The strongest expression was seen in the extraembryonic region, in particular in the visceral and parietal endoderm, which showed nuclear localization of the fusion protein. Expression levels were heterogeneous in the extraembryonic ectoderm and trophoblast, whereas in a small proportion of cells in these tissues, β -Gal was seen in both the nucleus and cytoplasm. Weaker expression that was clearly not restricted to the nucleus was apparent in the epiblast. During gastrulation, extraembryonic β -Gal activity remained strong and widespread, whereas expression in the embryo proper became progressively restricted (Fig. 2C). By late gastrulation, stained cells were seen in the streak and node, while expression was sharply downregulated in nascent mesoderm (Fig. 2D). In the remaining ectodermal layer, β -Gal activity was restricted to the neurepithelium of the head folds. Cells in the neurectoderm and the streak showed both cytoplasmic and nuclear β -Gal activity.

At 9.5 d.p.c., expression remained moderately high throughout the neurectoderm (Fig. 3A) and the late primitive streak (Fig. 3B). Strong de novo activation of mesodermal expression occurred in the presomitic mesoderm, concordant with the formation of new somites (Fig. 3B and C). β -Gal activity was restricted to the anterior half of the condensing and most recently formed somites while it seemed weaker and uniformly distributed in more differentiated somites (Fig. 3A and C).

At 12.5 d.p.c., β -Gal activity predominated in the nervous system and developing muscles (Fig. 3D and E). Interestingly, expression in the tail bud was particularly high in the chondroneural hinge (CNH), a region encompassing the most posterior part of the neural tube and shown to contain progenitor cells for elongation of the axis (4) (Fig. 3F).

Reporter expression at 15.5 d.p.c. was similar to that seen at 12.5 d.p.c., with the highest β -Gal levels in parts of the nervous system and the developing musculature (Fig. 3G and H). Weaker expression was observed in the liver. In the central nervous system, expression was seen in the neural progenitors in the ventricular zone of the cortex (Fig. 3H) and also at higher levels in more differentiated progeny (Fig. 3H and I). While β -Gal localization was mainly cytoplasmic in the neural tissue (Fig. 3I), it was nuclear in all the musculature examined (Fig. 3J) and in the subset of cells that showed expression in the liver (Fig. 3K). Strong cytoplasmic β -Gal activity was also observed in deposits of brown fat, the submandibular gland, the gut epithelium, and epithelial structures in the kidney and testis (data not shown).

Molecular characterization of the E148 integration. Fluorescent in situ hybridization (FISH) and Southern blot analysis revealed a single vector insertion at the proximal region of chromosome 3 (Fig. 4 and data not shown). A unique endogenous sequence (154 bp), which corresponds to a single open reading frame fused in frame with β geo, was identified by 5'

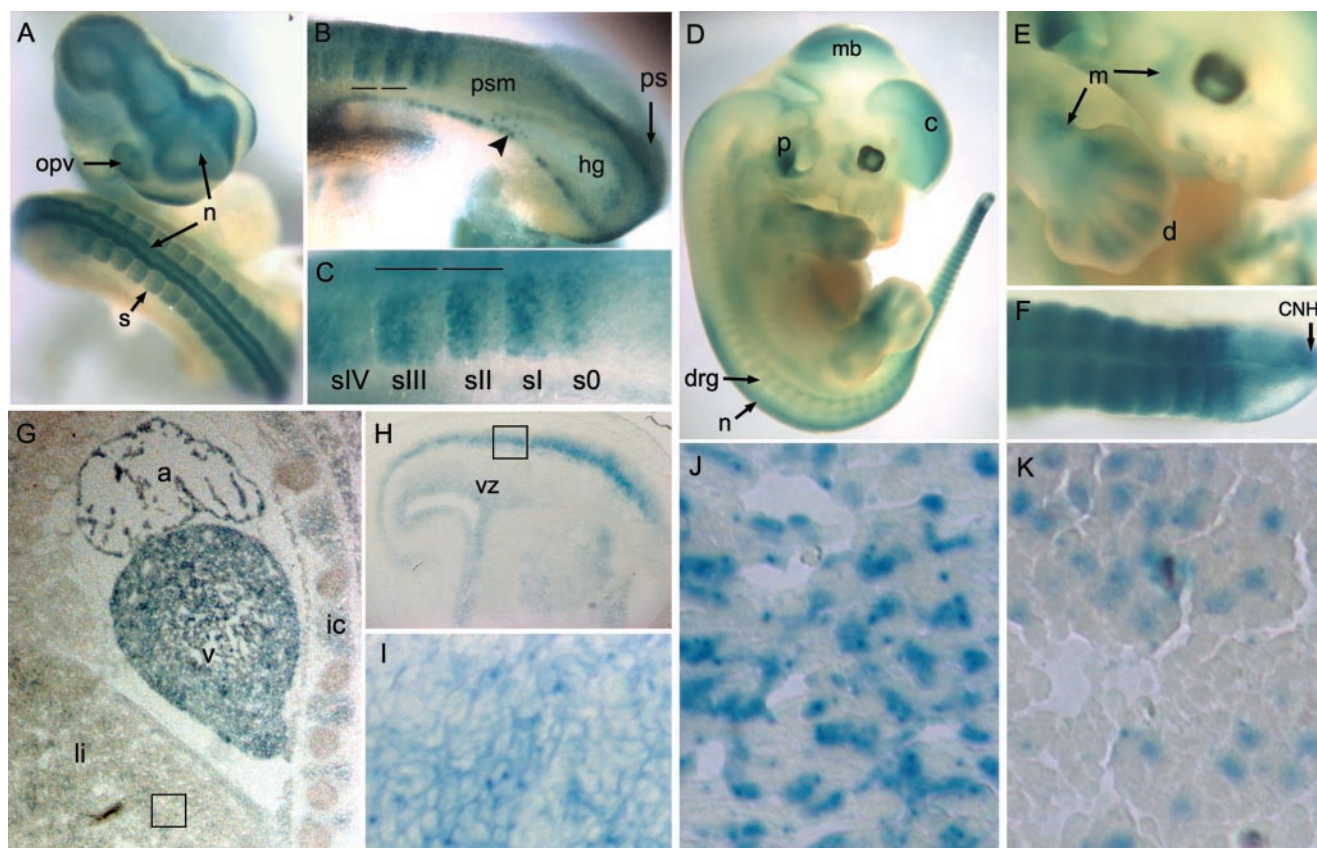


FIG. 3. Reporter expression in E148 mice during organogenesis and later fetal development. (A) A 9.5-d.p.c. embryo showing strong β -Gal expression in the developing neural tube, optic vesicle, and somites. (B) Enlarged view of the posterior end of a 9.5-d.p.c. embryo, showing expression in the ectodermal layer of the primitive streak. De novo upregulation of β -Gal expression occurs in somites emerging from the presomitic mesoderm. Horizontal lines in panels B and C indicate the limits of somites sII and sIII. A subset of cells throughout the gut, overlapping with the presumed location of primordial germ cells at this stage (16), show intense staining (arrowhead). (C) Enlarged view of the presomitic mesoderm and posterior somites. β -Gal activity is present in the anterior compartment of the nascent somite (s0) and three most recently formed somites (sI to sIII). In more mature somites (sIV and panel A), the level of β -Gal activity is lower and more evenly distributed throughout the somite. (D) Lateral view of a 12.5-d.p.c. embryo showing expression in the cerebral cortex and other neural tissue, in the pinna of the ear, and in somites and limb buds. (E) Enlargement of the embryo shown in panel D. β -Gal expression is high in developing muscle and in cells surrounding the digits. (F) Tail of a 12.5-d.p.c. embryo showing β -Gal activity in somites and the CNH of the tail bud. (G) Detail from a sagittal section of a 15.5-d.p.c. embryo. Expression is strong in the heart and in intercostal muscles, and more moderate expression is found in liver. The box shows the approximate region enlarged in panel K. (H) Sagittal section through the head of a 15.5-d.p.c. embryo, showing β -Gal activity in the ventricular zone, containing progenitors for the cortex, and in a subset of more differentiated cells. The box indicates the approximate region enlarged in panel I. (I) Differentiating cells in the cerebral cortex show cytoplasmic localization of β -Gal protein. (J) In muscle cells, expression is strong and localized primarily to the nucleus. (K) Liver cells show weaker nuclear expression. n, neural tube; s, somite; opv, optic vesicle; psm, presomitic mesoderm; ps, primitive streak; hg, hindgut; c, cerebral cortex; mb, midbrain; drg, dorsal root ganglia; p, pinna; m, muscle; d, digit; a, atrium; v, ventricle of the heart; li, liver; ic, intercostal muscle; vz, ventricular zone.

RACE-PCR. In the initial database searches, this sequence showed homology only to ESTs indicating that integration had occurred into a novel gene. The contigs made with mouse and human ESTs aligned to unique genomic sequences (Ensembl) in syntenic regions of mouse chromosome 3 and human chromosome 4. These findings indicated that these ESTs, including the E148 endogenous sequence, correspond to single orthologous genes in humans and mice. While our study was in progress, a human cDNA clone corresponding to this gene was identified in a two-hybrid screen for proteins interacting with the tumor suppressor pVHL and was named *Jade-1* (69). Hereafter, we use the names *mJade1* and *JADE1* to define the gene interrupted in E148 mice and its human orthologue, respectively, while the term *Jade1* designates both orthologues.

The alignment of human and mouse ESTs and cDNAs to the genomic sequences generated a map of exons (Fig. 5A) and indicated the presence of alternative exons at both ends of *Jade1*. The production of alternative transcripts was further supported by the detection of multiple bands in Northern blots (see Fig. 7B and reference 69 for *JADE1*). A major transcript of approximately 6 kb was detected, which corresponds well with the size of the transcript estimated by alignment of all ESTs. Some faint bands suggesting the presence of minor, lower-molecular-weight products were also seen. One of these smaller transcripts (~3.5 kb) coincides with the size of the *JADE1* cDNA clone (accession no. AF520952) isolated by Zhou et al. (69).

Predicted translation starts at the first exon common to all

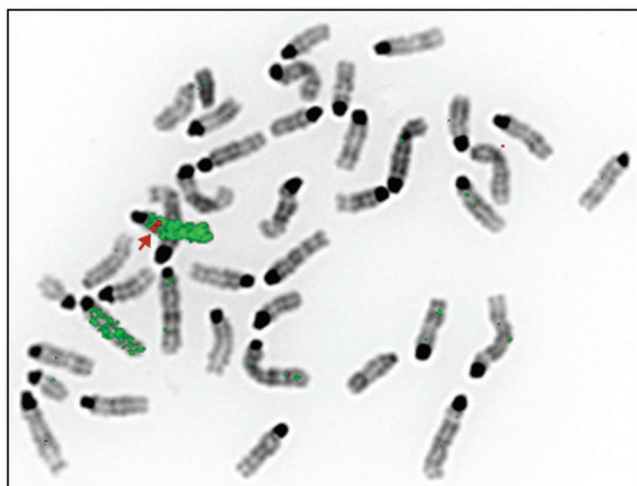


FIG. 4. Cytogenetic mapping of E148 integration. FISH in metaphase chromosome spreads of E148 ES cells with a probe specific to the gene trap vector detects a single chromosomal integration site on the proximal region of chromosome 3 (red). This location was confirmed by using a chromosome 3-specific paint (green).

transcripts (exon 2). The major 6-kb transcript is predicted to encode a protein of 834 amino acids and approximately 94 kDa while the 3.5-kb transcript results in a truncated form of the protein (510 amino acids, 58 kDa). Here, we designate the short and long protein isoforms *Jade1S* and *Jade1L*, respectively. An ~61-kDa band corresponding to *Jade1S* was seen in Western blots with an antiserum raised against a 20-amino-acid peptide in the C-terminal sequence of both human and mouse *Jade1S* (69). Although the upper part of this blot was not shown, Zhou et al. mentioned the presence of an additional band at 95 kDa (see discussion in reference 69), supporting the existence of the long isoform.

Insertion of the vector occurred in the third intron leading to the production of a 47-amino-acid truncated protein (Fig. 5). PCR amplification of the integration site with primers hybridizing to the 5' intronic sequence flanking this site and *lacZ* provided further confirmation that the gene trap vector had inserted into *mJade1* (data not shown).

***Jade1* belongs to a subfamily of PHD zinc finger proteins.** *Jade1L* contains two PHD zinc finger domains, two strong candidate PEST motifs, and a bipartite nuclear localization signal (NLS) (Fig. 5B). Interestingly, this NLS is not included in either *Jade1S* or the truncated *mJade1*- β -Gal protein produced by the gene trap allele. Conservation is high throughout the amino acid sequence of human and mouse *Jade1*. Strong conservation, albeit at lower levels in the C-terminal third of the sequence, also exists between human and mouse *Jade1* and a zebra fish *Jade1* orthologue (Fig. 5B).

Significant similarity to *Jade1* was also detected in ESTs and cDNAs corresponding to genomic sequences mapping to mouse chromosomes 11 and X and syntenic regions of human chromosomes 5 and X, respectively. We designate these genes *Jade2* (mouse chromosome 11 and human 5) and *Jade3* (mouse and human chromosome X). The overall exon-intron organization of these genes is similar to that of *Jade1*. Extensive homology, extending well beyond the PHD zinc finger do-

main, was observed between the deduced amino acid sequences of *Jade1*, 2, and 3. (Fig. 6A and data not shown). The strongest conservation can be seen in the N-terminal part and, in particular, the interfinger domain. Shorter amino acid stretches are identical in the region C-terminal to the PHD fingers. It is therefore probable that these highly conserved domains and C-terminal motifs characterize and define a novel subfamily of PHD zinc finger proteins. All three *Jade* members were also identified in *Fugu rubripes* while a single *Jade* homologue was found in the complete *Ciona intestinalis* genomic sequence. Finally, only two *Jade* genes were found to date among zebra fish ESTs. Figure 6B shows a phylogenetic tree constructed by comparison of members of this subfamily in different chordates.

Phenotypic analysis. To investigate any phenotypic defects resulting from homozygosity for the E148 gene trap insertion, transgenic F_1 offspring were backcrossed to C57BL/6 animals for several generations and the resulting heterozygotes were intercrossed. The results of this analysis revealed that homozygous animals are viable and fertile, showing no obvious morphological abnormalities compared to their wild-type and heterozygote littermates. However, the number of homozygotes obtained at weaning age was significantly less than expected according to the Mendelian ratio (28 of 184, or 15.2%; $0.05 > P > 0.01$). Further examination is therefore required to exclude a more subtle or genetic background-specific effect of gene trap integration on the viability of E148 homozygous mice.

Endogenous transcripts are disrupted by the gene trap integration. One possible explanation for the absence of overt phenotypic defects in E148 homozygous mice is that the gene trap integration has failed to generate a null allele of *mJade1*. Inefficient use of the polyadenylation signal and splice acceptor of the gene trap construct may result in splicing around the vector, such that sufficient quantities of wild-type transcript are generated to sustain normal function (9, 33, 43, 58). To test this possibility, a Northern blot was performed on total RNA prepared from 13.5-d.p.c. embryos of an intercross litter. As can be seen in Fig. 7B, a probe complementary to the *mJade1* sequence, 3' of the gene trap integration, fails to detect the major 6 kb and shorter *mJade1* transcripts in homozygous embryos. The absence of trace quantities of wild-type transcript in homozygotes was further confirmed by RT-PCR with two sets of primers, each amplifying a sequence specific to the wild-type or trapped allele (Fig. 7C). These findings indicate that endogenous transcripts are efficiently disrupted by the gene trap insertion.

DISCUSSION

We have characterized a gene trap insertion isolated in an in vitro screen designed to enrich for mutations in genes expressed in the primitive streak and tail bud during mouse embryogenesis. Reporter expression is detected in these structures as well as in a subset of other tissues during development. We have shown that the E148 mouse line carries a mutation in *mJade1*, encoding a PHD zinc finger protein. A short *Jade1* isoform, encoded by a human cDNA clone previously identified, was shown to interact with the tumor suppressor pVHL (69). We demonstrated that integration of the vector efficiently

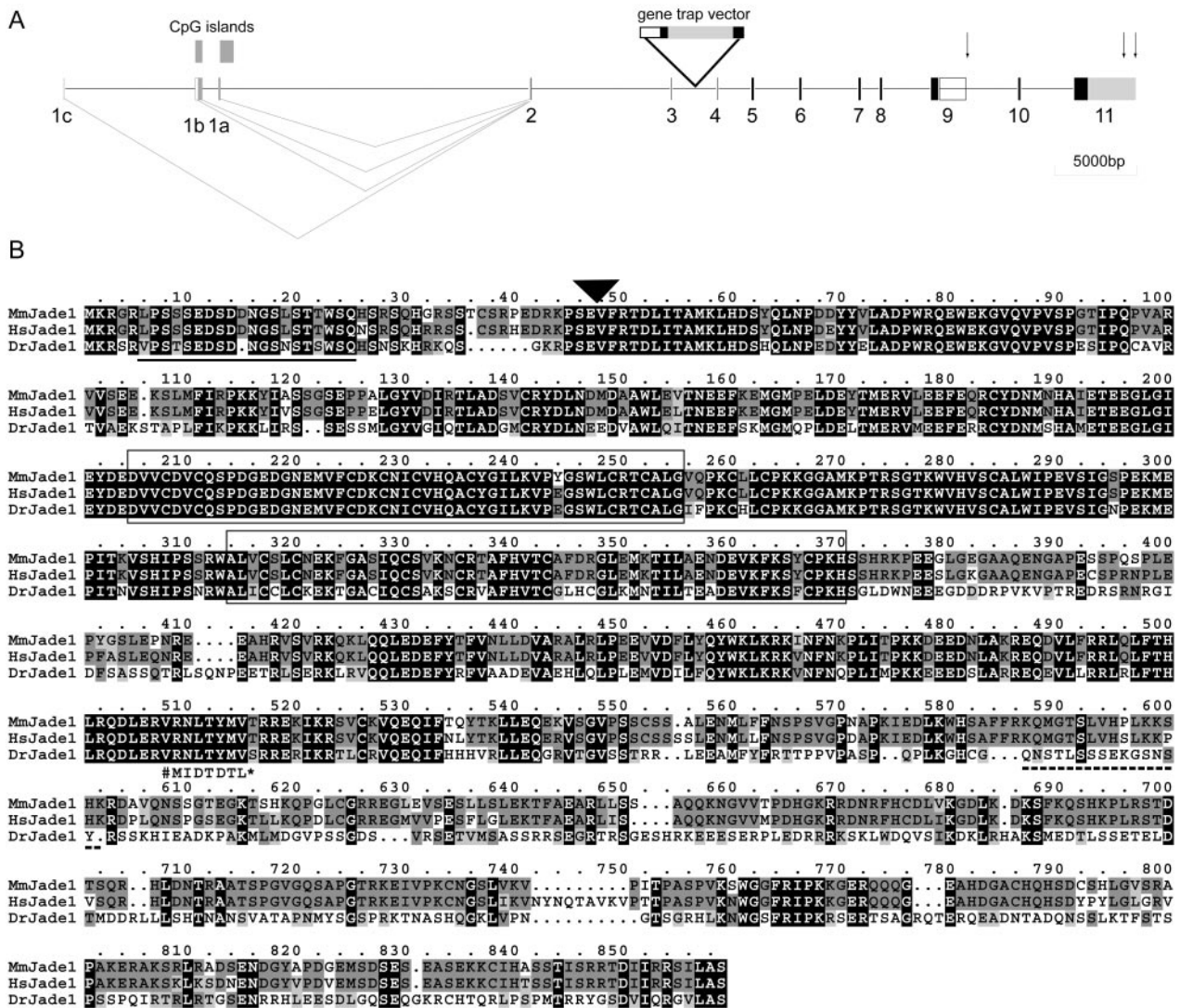
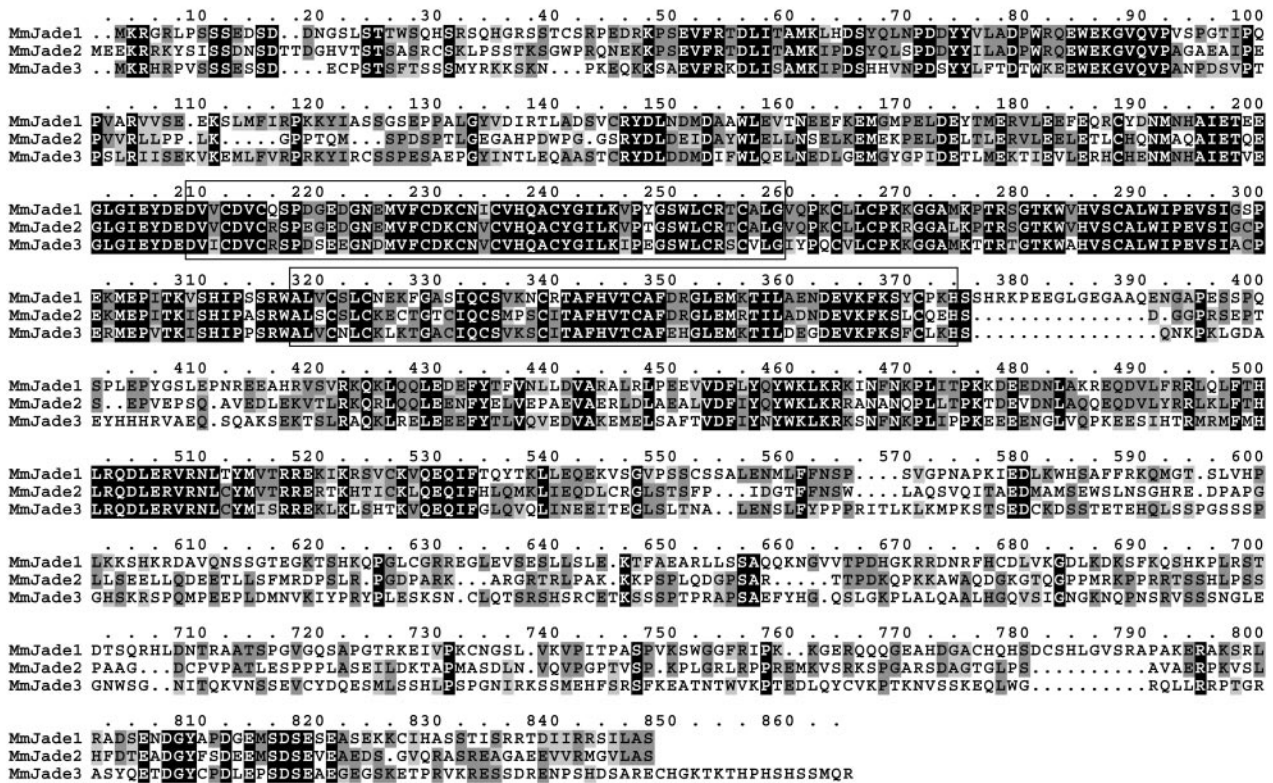


FIG. 5. *Jade1* gene structure and multiple-protein alignment of mouse, human, and zebra fish *Jade1*. (A) Mouse *Jade1* is encoded by 10 exons (black boxes 2 to 11). The gene trap insertion occurred between exons 3 and 4. Noncoding exons are shown in numbered grey boxes. Potential polyadenylation signals are indicated by arrows. The box outlined at the end of exon 9 indicates an alternative 3' untranslated region sequence that would give rise to a ~3.5-kb transcript and the short *Jade1* isoform (*Jade1S*), equivalent to the published human *Jade1* protein (69). EST and cDNA alignments indicate that at least three alternative 5' noncoding exons (1a to 1c) are used. A promoter scan identified two putative promoter regions in proximity to exons 1a and 1b that coincide with CpG islands. (B) Multiple alignment of mouse *Jade1* (Mm) with the putative *Jade1* orthologues in human (Hs) and zebra fish (Dr) reveals extensive homology. Residues identical in all three homologues are filled black while those identical only in two of these and conserved amino acid substitutions are filled dark and light grey, respectively. The PHD finger domains are boxed. A potential bipartite NLS is indicated by a dashed underline. Although this NLS is not conserved in the zebra fish, two possible overlapping bipartite NLS are present at positions 635 to 651 and 648 to 664 of Dr*Jade1*. Two putative PEST sequences (PESTFIND score > 10) found in both mouse and human *Jade1* are underlined in solid black. The position of the gene trap insertion is indicated by the black triangle. The published human *Jade1* protein sequence (GenPept accession no. AAM95612) is shorter than the sequence shown here; AAM95612 is identical up to the position marked # below the aligned sequences (corresponding to the end of exon 9) but is truncated at a stop codon (*) after 7 variant amino acids encoded by intron 9.

disrupts transcription of wild-type *mJade1*. Since the fusion protein produced by the trapped allele contains only a 47-amino-acid fragment of *mJade1*, it seems extremely unlikely that this protein retains any endogenous function. Therefore, we conclude that *mJade1*^{trE148ISCR} is a null allele. However, mice homozygous for the gene trap allele do not present any overt phenotypic defects. Database searches led to identification of a family of *Jade* genes conserved throughout vertebrates.

Posttranscriptional regulation of *Jade1* expression. Comparative analysis of the genomic and cDNA sequences suggested that primary *Jade1* transcripts in both mice and humans are subject to alternative splicing and polyadenylation. These posttranscriptional regulatory events result in production of several alternative mRNAs and at least two protein isoforms. In most of these transcripts, variation is limited to the sequence and/or size of untranslated sequences. Although these

A



B

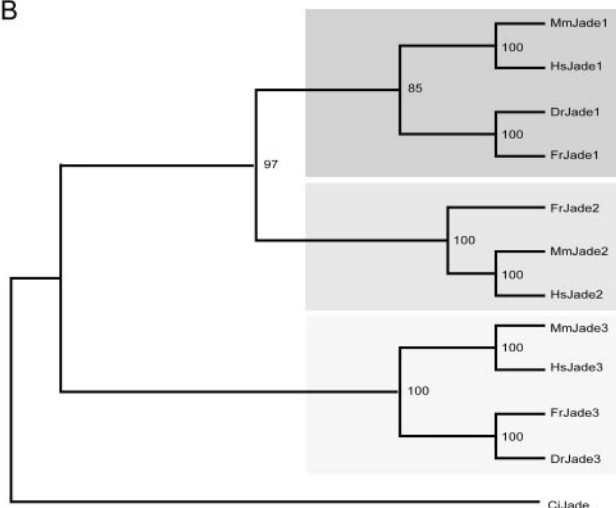


FIG. 6. The Jade protein family. (A) Multiple alignment of mouse Jade1 and the closest related mouse sequences (Jade2 and Jade3) identified in blast searches. Shading is the same as described in the legend to Fig. 5. The PHD fingers are conserved in all three family members (boxed), but there is also extensive conservation outside these domains. (B) A neighbor-joining tree of the Jade protein family. The tree was generated from an alignment of mouse (Mm), human (Hs), zebra fish (Dr), *Fugu rubripes* (Fr), and *Ciona intestinalis* (Ci) Jade sequences and rooted with the *C. intestinalis* sequence. Groups of Jade proteins are indicated by grey rectangles.

sequences do not contribute to the composition of the protein, they are known to regulate the stability and translation of transcripts (37, 39, 45, 56). The stability of gene products can also be regulated at the protein level by PEST sequences, such as these found in Jade1, that contribute to protein degradation

(5, 40). The study reported by Zhou et al. (69) shows that Jade1 is a short-lived protein, unless stabilized by pVHL. Since two strong candidate PEST sequences are present in Jade1L, while only one is found in Jade1S, these isoforms may show different degradation rates.

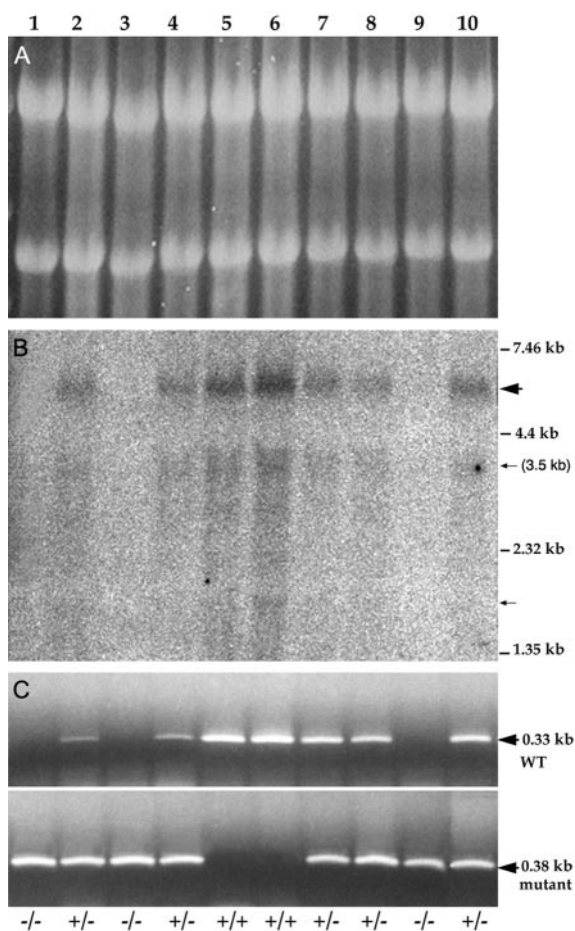


FIG. 7. Wild-type *Jade1* transcripts are absent in embryos homozygous for the E148 integration. Panels A and B show Northern blot analysis of total RNA prepared from 13.5-d.p.c. embryos (lanes 1 to 10) of a heterozygous intercross. The probe detects an *mJade1* sequence immediately 3' to the insertion site. (A) An ethidium bromide-stained gel loading control shows the presence of intact RNA. (B) In wild-type and heterozygous embryos a major ~6-kb transcript is present (wide arrow). Lower-molecular-size faint bands (indicated by small arrows) are also detected, indicating the presence of smaller minor transcripts, including one at 3.5 kb. No transcripts are detected in homozygous embryos. (C) RT-PCR analysis performed on the same RNA samples with primers from the endogenous *Jade1* sequence lying 5' and 3' to the E148 insertion (top panel). A wild-type band is detected in all heterozygous and wild-type embryos. A second RT-PCR with the same 5' primer and a 3' primer specific to the gene trap vector sequence amplifies a band in all heterozygous and homozygous embryos (bottom panel). No wild-type product is detectable in homozygous mutant embryos (lanes 1, 3, and 9).

The subcellular localization may also vary between *Jade1* isoforms. Immunohistochemistry with an antibody that potentially recognizes both isoforms revealed a differential nuclear or cytoplasmic localization of this protein in a number of cell types (69). The *mJade1*- β -Gal reporter was also localized to the nuclei of certain cell types, although this fusion protein does not include any sequence known to function as an NLS. It seems, therefore, that the short amino acid sequence (47 amino acids) at the N terminus of endogenous *mJade1*, present in the *mJade1*- β -Gal protein, is sufficient for its transport to the nucleus, at least in these cell types. However, the bipartite

NLS, included only in the predicted *Jade1L*, may direct a more exclusively nuclear localization of this isoform.

Molecular characteristics of the *Jade* family. The nuclear localization of the *mJade1*- β -Gal reporter and the presence of PHD zinc fingers in all *Jade* family members indicate that these proteins may regulate gene expression. The function of the PHD zinc finger domain, characterized by a Cys₄-His-Cys₃ pattern, is still not known. However, PHD fingers were found in several proteins implicated in chromatin-mediated transcriptional regulation. These include members of the *Drosophila melanogaster* Trithorax and Polycomb group genes known to regulate transcription of homeotic genes (14, 32), HRX, a human Trithorax homologue implicated in acute leukemia (15, 54), and the imprinting regulator DNMT3L (1). Another characteristic shared by many PHD finger-containing proteins is that they function as part of multicomponent complexes, raising the possibility that like the closely related LIM domain (46), PHD fingers are involved in protein-protein interaction. However, interaction of *Jade1* with pVHL was shown to occur independently of the PHD finger domains and is likely to be mediated by the interfinger region or the amino terminus of *Jade1* (69). Interestingly, the longest contiguous stretches of amino acid identity outside the PHD finger domains were found in these two regions of *Jade* family members. Thus, pVHL may interact with all three *Jade* proteins.

Interaction with pVHL and potential role of *Jade1* in placental vasculogenesis. Endogenous *Jade1S* levels were shown to increase in the presence of pVHL in 293T17 human embryonic kidney cells due to the prolonged half-life of *Jade1S* (69). pVHL functions as a tumor suppressor, and germ line mutations of the VHL gene are the cause of von Hippel-Lindau disease, a hereditary cancer syndrome in humans (reviewed in references 19 and 31). At the molecular level, pVHL seems to be involved in the control of diverse processes. While stabilization of *Jade1* was a new function attributed to pVHL (69), other better studied functions include targeting of specific proteins for polyubiquitination and subsequent degradation (29, 36) and, at least indirectly, regulation of gene expression (6, 8, 25) and mRNA stability (13, 18, 28). This functional heterogeneity is supported by the observation that pVHL forms multimeric complexes that may contain different partners. Furthermore, like *Jade1*, pVHL was shown to be differentially localized to the cytoplasm and nuclei of cells (27, 67). Targeted inactivation of VHL in mice results in embryonic lethality due to defective placental vasculogenesis (12). Consistent with this finding, expression of the angiogenic vascular endothelial growth factor is greatly reduced in pVHL-deficient placentas. The strong expression of the *mJade1*- β -Gal reporter in the progenitors of placental components (extraembryonic ectoderm and trophoblast) suggests that *Jade1* may play a role in the induction of placental vasculogenesis through its interaction with pVHL.

Embryonic expression of *Jade1*. The fusion *mJade1*- β -Gal protein was also expressed in many regions where multipotent or tissue-specific progenitors reside. During early embryogenesis, these sequentially include the pre- and early streak epiblast, the primitive streak and node, and finally, their descendant, the CNH of the tail bud. A balance between maintenance of these progenitor populations and their controlled differentiation and migration seems to be essential for the correct

specification of the different embryonic lineages and elongation of the A/P axis (62, 64–66). The expression of *mJade1* in these regions indicates that this gene may be involved in these processes. The finding that pVHL is important for epithelial differentiation and seems to inhibit cell migration (20, 30) further supports this possibility. pVHL also plays an important role in neuronal differentiation of central nervous system progenitor cells (21, 34). Interestingly, *mJade1*- β -Gal expression was seen in neural progenitors in the ventricular zone of the brain. Expression in a variety of stem cell types is also indicated by the isolation of *Jade1* cDNAs from hematopoietic, mesenchymal, and trophoblast stem cell libraries.

During organogenesis stages, expression of *mJade1*- β -Gal is strongly activated in stripes localized to the anterior compartment of the condensing and newly formed somites. This pattern is reminiscent of genes involved in segmentation of the paraxial mesoderm and determination of A/P identity, such as *Mesp2* and genes of the HoxD complex (38, 42, 68). Interestingly, global changes in chromatin structure of Hox complexes in both the primitive streak and tail bud and the nascent somites have been suggested to account for the sequential activation of progressively more 3' genes in the clusters as axial elongation proceeds, thus conferring successively more posterior identity upon somites (23, 24). Therefore, *Jade1* may function in the activation and/or repression of Hox complex genes via modulation of chromatin structure.

The high degree of conservation through vertebrate species argues for an essential function of *Jade1*. The absence of an overt phenotype could therefore indicate a functional compensation by other Jade family members. Examination of the libraries from which ESTs corresponding to *mJade2* and *mJade3* were isolated shows that these homologues are coexpressed with *mJade1* in a subset of tissues. In particular, *mJade3* is expressed in the early organogenesis stage embryo and an *mJade3* cDNA clone was isolated in a screen for genes involved in breast cancer (50). Thus, compound mutants lacking more than one Jade member may be required to reveal the function of these genes.

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